

161* Expression of virulence factors in methicillin resistant *Staphylococcus aureus* (MRSA) isolates from cystic fibrosis sputum

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Introduction: Lung colonisation with MRSA contributes to worse survival in patients with CF. This study aimed to determine differences in virulence factor expression in MRSA isolated from CF and non-CF patients, and from different CF centres. As oxygen can be limited in the CF lung, the impact of anaerobic (AnO₂) vs aerobic (O₂) growth on virulence factor production was also examined.

Methods: CF MRSA isolates were obtained from two paediatric (n=26) and one adult (n=10) CF centres and compared to non-CF MRSA isolates from paediatric intensive care (n=10) and non-CF patients undergoing decolonization (n=10). Isolates were characterised by PFGE, *agr*, *spa* and *SCCmec* typing. Levels of delta-haemolysin (δ-hly), SpA and TSST production were determined after O₂ and AnO₂ growth, by a semi-quantitative haemolysis assay (δ-hly) and Western Blotting (SpA and TSST).

Results: Isolates from each centre formed related pulsotypes. Levels of δ-hly were higher after AnO₂ vs O₂ growth [OD: 0.21 vs 0.15 respectively, (p=0.018)] in CF related MRSA, and significantly higher than non-CF MRSA, either grown in AnO₂ or O₂ conditions [OD: 0.02, p≤0.0001]. Levels of SpA were higher following O₂ vs AnO₂ growth in CF-MRSA, but not in non-CF MRSA. TSST was increased under both AnO₂ and O₂ conditions by CF isolates only.

Conclusions: MRSA cultured from patients in CF centres reflect those currently in circulation in the local environment; however, differences in key virulence factor production were observed between CF and non-CF MRSA. Further work investigating potential dysregulation of the MRSA respiratory response system (*Srr*) and consequences of this for clinical outcome, are ongoing.

162* Identical obligate anaerobic bacteria in periodontal pockets and sputum of cystic fibrosis patients

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In the lungs of almost every patient with CF, facultative and obligate anaerobic bacteria exist. The source of these pathogens is unknown. We investigated if periodontal pockets may act as origin of the bacteria. In sputum and swabs from dental pockets of 17 CF patients and in dental pockets of 33 non-CF controls facultative and obligate anaerobes were identified using the Vitek[®], the Raid AnaII[®] and the Crystal[®] identification systems. Resistance patterns were determined by e-test for ceftazidime, piperacillin/tazobactam, meropenem, azithromycin, metronidazole, colistin, and clindamycin. Genotyping was performed using pulsed field gel electrophoresis. In 8 out of 17 CF patients (47%) identical facultative anaerobes (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*) were detectable in sputum and in dental pockets. In every single CF patient (100%) identical obligate anaerobes (*Clostridium*, *Staphylococcus*, *Peptostreptococcus*, *Propionibacterium*, *Wolinella*, *Veillonella* spp.) were found in both compartments. Identical species were found in dental pockets of the controls. Resistance patterns were similar in CF patients and controls. Genotyping in three patients showed the same strains in CF sputum and dental pockets. Identical facultative and obligate anaerobic bacteria in dental pockets of CF patients suggest a relation between both compartments: (1) bacteria from the lungs may contaminate the dental pockets, or (2) the pockets may be the sources of CF lung infections. The simultaneous finding of the same bacteria with similar resistance patterns in dental pockets of healthy controls stands in favour of the second assumption.

163 Molecular investigation of *Herbaspirillum* as a novel pathogen in cystic fibrosis (CF) sputae and its *in vivo* virulence in the *Galleria mellonella* model

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This study investigated the phenotypic and genotypic identification, genetic relatedness and potential virulence of a cluster of *Herbaspirillum* spp. in a CF clinic. An invertebrate *Galleria mellonella* model was used to investigate the virulence of CF pathogens. DNA extracts were sequenced and used in RAPD, integron and 16sRNA PCR. 10 isolates were confirmed as *Herbaspirillum* spp. by genomic sequencing and all were indistinguishable by RAPD PCR. There was negligible evidence of integron presence in the different strains.

The pathogenic investigations found virulence to be considerably less than that seen with *Pseudomonas aeruginosa* and more equivalent to *Streptococcus mitis* in the *Galleria* model. The clinical significance may be greater as a co-pathogen. The correct identification of the bacterial species profoundly defused both the clinical significance and the infection control risk in the CF clinic by confirmation that the outbreak strain was not *B. cepacia*. This study suggests human transmissibility of *Herbaspirillum* spp. for the first time in a clinical setting. The data show low levels of virulence *in vivo* in the *Galleria* model, however its significance in the CF microbiome merits further study.

164 Cystic fibrosis bacterial pathogens and *Aspergillus fumigatus* biofilm interactions

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Aspergillus fumigatus is often isolated from the lungs of cystic fibrosis (CF) patients, but unlike in severely immunocompromised individuals the potential for the organism to become invasive rare and the mortality rates are therefore low. This suggests that competition from bacteria within the CF lung may be inhibitory. Interactions between *Pseudomonas aeruginosa* and *Aspergillus fumigatus* have shown cross talk between these organisms which can impact on *A. fumigatus* biofilm formation. This study examines the effect of key CF pathogens *Burkholderia cenocepacia* and *Staphylococcus aureus* on *A. fumigatus* biofilm formation and how this relates to a 'snap shot' audit of adult CF patients in the West of Scotland. *In vitro* six clinical representative strains of both *B. cenocepacia* and *S. aureus* were used. *A. fumigatus* was inhibited by direct contact with *B. cenocepacia*, but had no effect on preformed biofilm. A secreted heat-stable soluble factor was also shown to exhibit biofilm inhibition this is similar in pattern to the results seen in *P. aeruginosa*. *S. aureus* however did not show any effect of *A. fumigatus* growth in any phase. In patients mixed biofilms particularly mould and either *P. aeruginosa* or *B. cenocepacia* resulted in poorer lung function (% predicted FEV) than patients without moulds. Overall, this suggests that small diffusible and heat-stable molecules in both *P. aeruginosa* and *B. cenocepacia* may be responsible for the competitive inhibition of filamentous fungal growth in polymicrobial environments such as the CF lung however established fungal biofilms allow provide a matrix for bacterial colonisation.